BIOLOGICALS FOR THE CONTROL AND THERAPY OF VIRUS DISEASES[1]

RODERICK MURRAY

Division of Biologics Standards, National Institutes of Health, Bethesda, Maryland

INTRODUCTION

It is a rare week during which one does not see in the popular lay or medical press some reference to a "new vaccine." The news is sometimes carried in the financial section. If it is a "new vaccine" against a virus disease, the story is more glamorous and attractive as a news item; but in fact the public seems to find the very idea of a vaccine a fascinating one!

The field of virology is expanding with great vitality, and information on the nature and properties of viruses is accumulating at a phenomenal rate. The new viruses being identified in large numbers represent only part of this information, as some of the presentations made at this conference have shown. It is in connection with the discovery of these viruses that reports of the possibility of development of new vaccines stimulate the imagination.

Once the virus implicated as the cause of a particular disease has been isolated and can be propagated by suitable means, the development of a vaccine becomes a possibility. However, it is a far step from the isolation of a virus or even from the demonstration that it is possible to prepare an antigen from it to the point at which a useful vaccine may become available. A new vaccine only becomes a reality when its usefulness and safety have been proved—that it is satisfactory in use and can be given safely to

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large numbers of people—or, in the statutory phrase, that it is safe and potent (United States Code, 42 U.S.C. 262).

RECENTLY INTRODUCED VIRAL VACCINES

The fact is that during the course of the past 9 years, which have been active years of the tissue-culture era, only five new virus vaccines have been licensed in the United States (Code of Federal Regulations, 42 C.F.R. 73): (i) inactivated poliomyelitis vaccine (Salk vaccine); (ii) adenovirus vaccine (types 3, 5, and 7); (iii) live poliovirus vaccine (three individual types and a trivalent preparation); (iv) measles vaccine, live, attenuated; (v) measles vaccine, inactivated.

It will be noted that these five vaccines are only against three named virus diseases. Actually, seven viruses are involved because the three poliovirus types and the three adenovirus types represent separate vaccines. On this basis, it would seem that there might be little to discuss. Unquestionably, many new vaccines will be developed following the extensive and fruitful research which is going on at the present time. Vaccines against a variety of respiratory infections and against rubella are distinct possibilities. Hopefully, there could also be some improvement in existing vaccines.

It might be useful to discuss briefly some of the problems associated with the development of new vaccines.

First of all, most of the newer vaccines are produced in tissue-culture preparations. This fact alone gives rise to a number of the problems encountered with new vaccines. In this regard, the experience gained in the development and

manufacture of Salk vaccine has been of great value in approaching, and in anticipating the problems of new virus vaccines. The main lessons learned here were: (i) the scope and sensitivity of the safety tests which must be employed; (ii) the need to have a standardized manufacturing system which can consistently produce a series of lots of vaccine which satisfactorily meet prescribed standards; and (iii) the need for the accumulation of rather substantial field experience with a product before it is used on a large and unrestricted scale. The relative freedom from difficulty with which measles vaccine and live poliovirus vaccine (Sabin) were introduced was in large measure, I believe, related to this experience.

Strains

The strain selected for vaccine production should be capable of producing a product of high immunogenicity. In the case of inactivated vaccines, the height of the virus titer which can be achieved in the tissue-culture system selected is of great importance in the development of a vaccine of satisfactory antigenicity, since this is directly related to the amount of antigen. Unless high titers can be achieved, the antigenicity of the product may be borderline, and this situation brings with it a whole series of undesirable production problems. Losses due to tissue-culture contamination and failure, difficulties with the inactivation process, questionable stability of the final product, and control testing problems which are difficult to resolve present themselves more frequently with materials of low titer than in the case of strains which propagate rapidly to high titer.

Choice of a Propagation System

A wide variety of tissue-culture systems are now used experimentally, and will undoubtedly be used in the future; but thus far the systems used in the production of vaccines which have been licensed are limited. The widest experience has been with primary monkey kidney tissue culture, with cells from Rhesus, cynomolgus, and cercopithecus monkeys, and with chick-embryo tissue culture. Other primary cell systems which may also be used but have so far only been used in investigative situations are tissues from domestic or laboratory animals; these include canine, bovine, and guinea pig cells. Primary tissue-culture cells have thus far been the only practical sources for the manufacture of inactivated vaccines because of the large amounts of virus required. The main disadvantage of their use is that many adventitious agents are encountered in their use (2, 4, 8). The occurrence of these agents gives rise to serious production and testing problems. The use of continuous cell lines which would provide a partial solution is beset with two main difficulties: (i) technology of supplying a sufficient quantity, particularly in the case of inactivated vaccines; and (ii) acceptance of such cell lines as being suitable for the production of vaccine, particularly of live vaccines, in view of the fact that the cells of such systems have the property of continuous growth (7).

Adventitious Agents

A great many adventitious agents have now been encountered in the course of tissue-culture work. Most of these represent merely testing and production difficulties of no significance to the safety of the final product since they can be detected quite readily by the test systems employed, and there can thus be an assurance that the final product is free from such contamination, as is the case with the analogous situation of bacterial contamination of bacterial products such as toxoids. Two agents have thus far presented special problems; these are SV40 and the agent of avian leucosis. Both are oncogenic in animals under certain conditions, and SV40 is particularly resistant to inactivation.

Sensitivity of Tests for the Presence of Virus After Inactivation

Perhaps the single most important procedure in determining the safety of an inactivated vaccine is the test for residual virus after inactivation. The tests used must therefore be adequate, both in terms of the sensitivity of the test system and the size of the sample tested to assure that the product is safe. In the case of vaccines produced from pathogenic viruses, the one piece of information which would be most helpful is lacking and unobtainable; this is the relationship between the smallest quantity of residual virus detectable by the system used and the amount which could infect a human being.

Special Problems Presented by Live Virus Vaccines

Because actual infection takes place as part of the process of immunization, the virus used in

the preparation of live virus vaccines must be rigidly controlled with respect to identity, freedom from other viruses, and any influences which might alter the characteristics of the virus in the final vaccine. Thus, there must be absolute identity of the seed virus used and strict control over the number of passages by which the virus in the final vaccine is removed from the virus used in field trials for the validation of the vaccine. In the case of yellow fever vaccine, the seed virus must be within a prescribed ten passages of a master seed and, in the case of live poliovirus vaccine, the number of passages permitted between the virus in the final product and that used for clinical trials is limited to five. These field trials should also demonstrate that the product is free from harmful effects in recipients or their contacts. Ideally, there should be a demonstration that the product will protect against actual disease; however, in those situations where the development of antibody has been shown to be virtually synonymous with protection, this demonstration can depend upon the development of adequate levels of antibody. Particular vigilance must be aimed at the detection of possible adventitious agents in live vaccines, since once these are present in a virus harvest there is no satisfactory way of removing them from the vaccine.

Once a live vaccine has demonstrated its acceptability, it is generally easier to administer than the inactivated vaccines, because a single dose is usually all that is required for primary immunization. There is also the advantage that potency can be determined in terms of a measurement of virus titer—a relatively simple and rapid procedure when compared with the elaborate animal potency test required for inactivated vaccines.

Multiple Antigen Products

Products which produce immunity to a wide variety of infectious diseases would be desirable from the point of view of those responsible for immunization programs and for the comfort of the patient. A great deal has been written of the possibility of combining a great many antigens into a single preparation and simultaneously producing immunity to a wide variety of diseases. This may be possible some day. At the present time combinations of diphtheria toxoid, tetanus toxoid, and pertussis vaccine are being success-

fully used. Unfortunately, difficulties multiply—particularly in relation to the potency of the material—and recent experience with the pertussis component of a so-called four-way antigen which includes inactivated poliomyelitis vaccine suggests that optimism for multiple antigen preparations should be restrained (1, 6). In the case of inactivated or killed vaccines, the cost and difficulty of assaying the potency of such preparations might well be very great. This is a matter which requires a great deal of further study, and these preparations might have a greater chance of actuality when antigens of relatively high purity become available.

The possibility of using a combination of live virus vaccines exists, providing the administration is simultaneous and the vaccines and the infections to which they give rise are compatible. The feasibility of using a combination of measles, yellow fever, and smallpox vaccine was recently demonstrated in a pilot study (5).

BIOLOGICALS FOR THERAPY

Although the title of this presentation contained the word "therapy," one must admit that the main significance of biological products in the field of viral infections is for prevention. Rabies vaccine has the distinction of being a therapeutic vaccine. Other than this, examples are hard to find, with the exception of human γ-globulin which, as is well known, has been used to passively protect against poliomyelitis, measles, hepatitis, and a variety of other infectious diseases. Its role in rubella is currently in dispute (S. R. Krugman, *personal communication*), but there are indications that γ-globulin prepared from the plasma of convalescent cases may provide a means of therapy for a wide variety of rare diseases which cannot be treated in other ways. The use of vaccinia γ-globulin for disseminated vaccinia is an example of the use of such a preparation (3).

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